A new subspecies of *Actias chapae* Mell, 1950 from southern Vietnam (Lepidoptera: Saturniidae)

Andrei V. Sochivko and Nikolai V. Ivshin

Andrei V. Sochivko, Akademika Volgina str., 31-1-72, Moscow, 117437, Russia; sotchivko@mtu-net.ru.

Dr. Nikolai V. Ivshin, The Laboratory of Comparative Genetics of Animals, Institute of General Genetics, Gubkina-3, Moscow, 117809, Russia; nickolai.ivshin@gmail.com.

Abstract: A new subspecies of *Actias chapae* Mell, 1950, *A. chapae* ***bezverkhovi*** **ssp. n.,** is described from southern Vietnam (type locality: Langbian plateau; holotype male deposited in coll. Dr. S. Naumann, Berlin, Germany, eventually in Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany). This silk moth can be distinguished from the nominotypical subspecies by a number of characters such as external habitus, genitalia morphology, and flying season. The DNA sequence of a 596 bp marker fragment of the mitochondrial COI gene is provided.

Keywords: Langbian plateau, characters, identification, description, mt-DNA.

Eine neue Unterart von *Actias chapae* Mell, 1958 aus dem südlichen Vietnam (Lepidoptera: Saturniidae)

Zusammenfassung: Eine neue Unterart von *Actias chapae* Mell, 1950, *A. chapae* ***bezverkhovi*** **ssp. n.,** wird aus dem südlichen Vietnam (Typenfundort: Langbian-Plateau; Holotypus Männchen in coll. Dr. S. Naumann, Berlin, später im Museum für Naturkunde der Humboldt-Universität zu Berlin, Deutschland) beschrieben. Diese Saturniidenunterart unterscheidet sich von der nominotypischen Unterart vom Fan-Si-Pan (nördliches Vietnam) in einer Reihe von Merkmalen wie genereller Habitus, Genitalmorphologie und Flugzeit. Die Gensequenz eines 596 DNA-Basenpaaren langen Marker-Fragments des Gens der mitochondrialen Cytochromoxydase, Untereinheit I (COI), wird vorgestellt.

Introduction

The history of the investigation of biological and morphological traits of the silkmoth *Actias chapae* Mell, 1950 was for a long time incomplete. The original description was based on two specimens (♀ and ♂) kept in Museum Alexander Koenig, Bonn, Germany. Several decades later the type material was revised, and the misidentifications were assessed (Nässig & Brechlin 1995, Wu & Naumann 2006); the nominal taxon *chapae* was fixed to the ♂ specimen, now being the lectotype. Its locality of origin was indicated as "Chapa, (Tonkinesische Hochalpen), 1500 m, Edinger [leg.]". There were no other details. This place is situated in northern Vietnam, near Chapa (= Sa Pa) village, Fan Si Pan (= Fansipan) mountains. The ♀ specimen earlier included into the type series of *A. chapae* was identified as a ♀ of *A. rhodopneuma* Röber, 1925 (Nässig & Brechlin 1995). The flight season data were obtained only many years later by the Russian entomologist Viktor Sinjaev who found *A. chapae* again at the type locality in November 1994, at 2350 m elevation a.s.l. The first ♀ of this beautiful moth was then among the collected specimens. The material was collected during 1994 and the following years, demonstrating the seasonal range of imagines of *A. chapae* between end of October and end of November. The records for *A. chapae* in the Fan Si Pan area were found at elevations from 2250–2350 m, only the ♀ was found at 1600 m (Wu & Naumann 2006, V. Sinjaev pers. comm.). See Fig. 1.

Later K. Morishita and Y. Kishida discovered a new habitat of *A. chapae* in China: Nanling Shan (Shan = mountains), Guangdong province (Morishita & Kishida 2000). These researchers and later also V. Sinjaev collected small series of material in Guangdong, Guangxi and Hunan provinces of China between the middle of November and middle of December at the following elevations: Jiucai Ling (Hunan), 700 m; Dayao Shan (Guangxi), 1600 m; Nanling Shan (Guangdong/Hunan border area), 1100–1850 m.

In early April 2007 a recent Russian entomological expedition to southern Vietnam started. The main samples of various insect groups were collected from the northern part of the Langbian plateau around Dalat town.

A narrow stripe of the tropical mountain forests still exists there and surrounds an area strongly transformed by man. The primary forests are under permanent stress due to regular fires, invasion of agriculture, logging, and construction companies (Goldammer 1992). Pine plantations substitute the forest in logged areas. It is rather ridiculous, but the efforts of local nature protection services are mainly targeted on preserving these plantations. A dry climate, combined with regular fires in these artificial biotopes speed up the death of the local tropical mountain forests — the habitat of *A. chapae*.

Mell (1950) assumed that the specimens of *A. chapae* found in autumn are representatives of a second generation. It is, however, quite unlikely that there is a spring generation in these rather well explored areas in northern Vietnam and China from where nominotypical *A. chapae* is recorded so far. There were so many expeditions and collecting efforts by Russian, Vietnamese, Japanese and Chinese collectors all year round that a possible second generation should long have been found. Also from the long lasting development of the preimaginal instars (Wu & Naumann 2006) this seems to be improbable.

Material and methods

All four ♂♂ of the new subspecies collected were attracted to a mercury light trap between 20:00 and 22:00 h at 1550 m altitude of the Langbian plateau around Dalat. The weather conditions were extreme enough: strong wind and fog, air temperature about 15° C.

© Entomologischer Verein Apollo e. V., Frankfurt am Main

DNA sequences

A few moths were investigated regarding molecular markers. One Chinese specimen of *A. uljanae* Brechlin, 2007 (paratype) and a specimen of *A. ningpoana* C. Felder & R. Felder, 1862 were taken as examples of accepted separate species. Among molecular markers a most popular one in taxonomic investigations is subunit I of the mitochondrial cytochrome oxidase gene (COI). For more information see the "Barcode of Life" initiative (Hebert et al. 2003a, b, Zakharov et al. 2007). This marker is sensitive enough to differentiate taxonomic groups at species and subspecies level. At the same time it is almost insensitive to intra-population variability.

The DNA of the moths was extracted using DIAtom™ DNA Prep kit (Izogen, Moscow). DNA samples were obtained from the legs of dried specimens. The primer pair LCO1490 and HCO2198 (see Tab. 2) was subsequently used to amplify a 596 bp fragment of the Cytochrome Oxidase subunit I gene. The amplification reactions were carried out in the final volume of 25 mkl with 20 pmol of each primer, 0.1 g of the isolated DNA and with the universal amplification kit GenePak@PCR Core (Izogen, Moscow). The polymerase chain reaction (PCR) was performed using a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, USA). PCR thermal regime consisted of one cycle of 1 min at 94°C; five cycles of 1 min at 94°C, 1.5 min at 45°C and 1.5 min at 72°C; 35 cycles of 1 min at 94°C, 1.5 min at 50°C and 1 min at 72°C and a final cycle of 5 min at 72°C (Folmer et al. 1994). The PCR products were identified using electrophoresis in 1% agarose gel (Sigma, United States). Each PCR product was sequenced in both directions on an ABI 3100 automated sequencer (Applied Biosystems) without preliminary purification. *A. chapae* DNA sequences obtained in this study will be submitted to NCBI GenBank. The DNA sequences were verified with ChromasPro, aligned and analyzed by mean of MEGA software version 3.0 (Kumar et al. 2004).

For the resulting base sequences, see Tab. 2.

Systematic part

Actias chapae bezverkhovi ssp. n.

(Figs. 3, 4, 7–9)

Holotype ♂ (Fig. 2): S. Vietnam, Langbian plateau, 40 km N Dalat, 1550 m, 9. iv. 2007, leg. Y. Bezverkhov, A. Sochivko & P. Oudovichenko; will be deposited in the collection of Dr. S. Naumann, Berlin, Germany and, eventually, in the collections of Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany.

Paratypes (in total 3 ♂♂, all same data as holotype): 1 ♂ in coll. A. Sochivko, Moscow, Russia; 1 ♂ in coll. S. Kovalenko, Moscow, Russia; 1 ♂ in coll. Y. Bezverkhov, Moscow, Russia.

Etymology. The subspecies is named after Yuri Alexeevich Bezverkhov, Russian statesman and organizer of current entomological research in Southern Vietnam.

Description and diagnosis

The comparative morphological characters are given in Tab. 1. The morphology details of the genitalia were taken from the article of Wu & Naumann (2006) and from our own collected material. As no ♀♀ of *A. chapae bezverkhovi* are known, the description deals only with ♂♂.

The other elements of wing pattern like form and position of dark medial band are rather variable and cannot be used to distinguish the subspecies.

Table 1: Comparison of the morphology of the two *Actias chapae* subspecies.

Male specimens Character	*A. chapae bezverkhovi*	*A. chapae chapae*
♂ genitalia: shape of the valves and harpe	The distal margin of the harpe is angular (Figs. 7–9)	The valves are less flat and more elongate; the distal margin of the harpe is round (Figs. 5–6)
♂ genitalia: dorsal tegumen (= 9th tergite) processes	The tegumen bears three dorsal processes (Fig. 8, lines); in the middle there is a small additional process, similar to a small "pseudouncus"	The tegumen bears only two processes (Fig. 6, lines)
Forewing: apex shape	The apex is slightly produced (Fig. 2)	The apex is round (Fig. 1)
Forewing: medial-discal eyespot border	Being formed by black scales, the border is darker in comparison with the closest veins; vein D2 exists (Fig. 4; for explanation, see Fig. 11)	Being formed by yellowish scales, the border is colored as dark as the closest veins. The short vein D2 is reduced completely (Fig. 3; for explanation, see Fig. 11)
Timing of adult generations	Spring generation (April) only known	Autumn generation (October/November) only known

Table 2: DNA base sequences (see text).

DNA	Base sequences
Primer LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'
Primer HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'
Subunit I of mitochondrial cytochrome oxidase gene (COI) of *Actias chapae bezverkhovi*	TTGGTCCAACCAATCATAAAGATATTGGAACTTTATATTTTATTTTTGGAATTTGATCAGGGATAGTGGGAACTTCTTTA AGCCTTCTTATTCGAGCTGAATTAGGAACTCCAGGTTCTTTAATTGGAGATGATCAAATTTATAACACAATTGTAACAGC TCATGCTTTTATTATAATTTTTTTTATGGTAATACCTATCATAATTGGAGGATTTGGAAATTGATTAATTCCTTTAATACT TGGAGCTCCAGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGATTACTTCCCCCATCTCTTATTCTTTTAATTTCT AGTAGAATTGTAGAAAATGGAGCTGGTACAGGATGAACAGTTTATCCACCTCTTTCTTCTAATATTGCTCATAGAGGAAC TTCAGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCTTCAATTTTAGGAGCTATTAATTTTATTACAACTATT ATCAATATACGAATAAATAACTTATCATTTGATCAAATACCTTTATTTGTATGAGCAGTTGGAATTACAGCTTTCTTACTC CTTTTATCTCTTCCTGTATTAGCTGGAGC

© Entomologischer Verein Apollo e. V., Frankfurt am Main

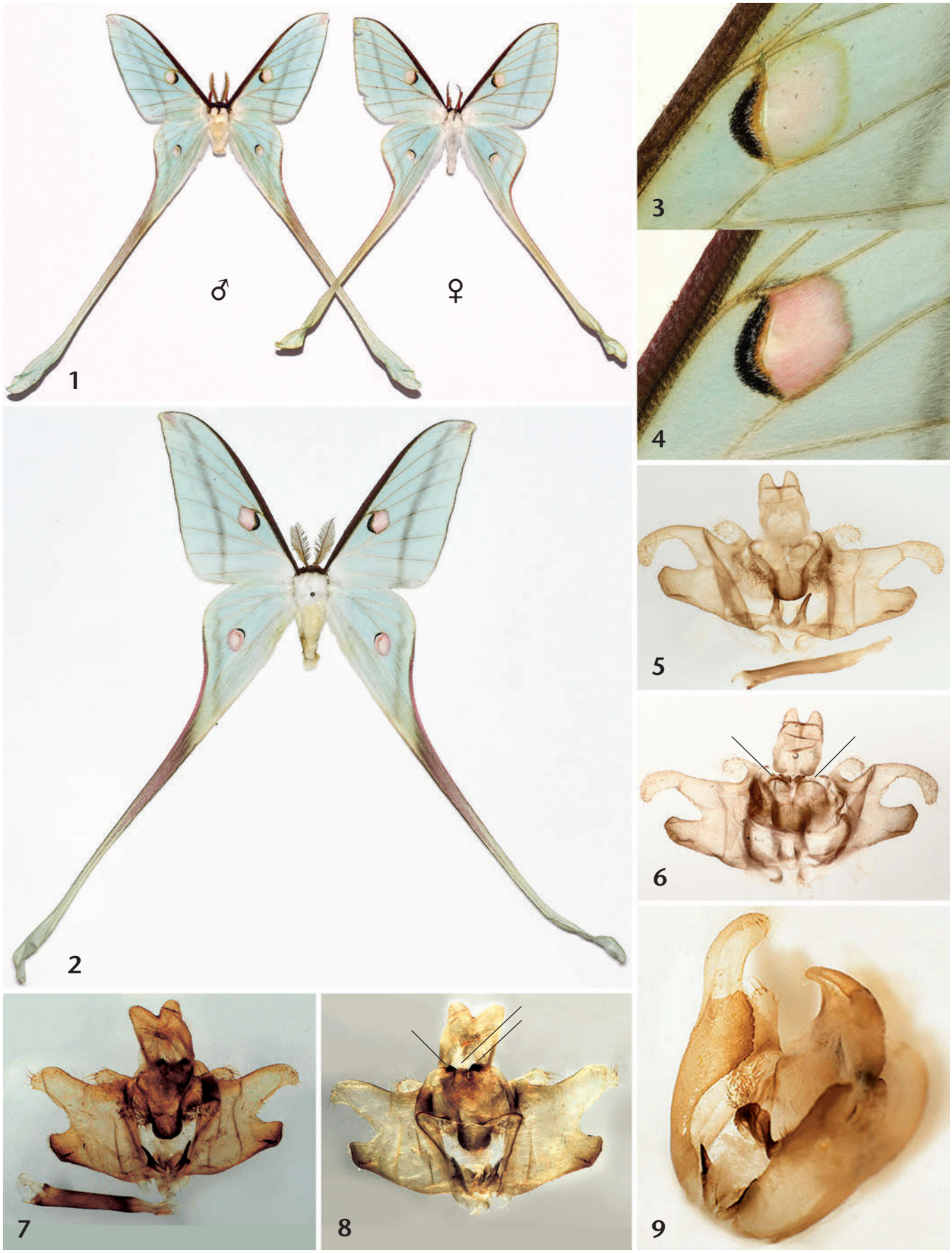

Fig. 1: Male and female of *Actias chapae chapae,* N. Vietnam, Fansipan Mt. (photo V. Sinjaev). — **Fig. 2:** Male holotype of *Actias chapae bezverkhovi*. — **Figs. 3–4:** Forewing eyespot in ♂♂ of *A. chapae*: **Fig. 3:** *A. chapae chapae*. **Fig. 4:** *A. chapae bezverkhovi* (compare Fig. 11). — **Figs. 5–9:** ♂ genitalia of *Actias chapae* subspecies. **Figs. 5–6:** *A. chapae chapae;* **Fig. 5** normal view from ventro-caudal side; **Fig. 6** opposite view from dorso-cephal side; lines to show the two dorsal tegumen processes. **Figs. 7–9:** *A. chapae bezverkhovi;* **Fig. 7** normal view from ventro-caudal side; **Fig. 8** opposite view from dorso-cephal side; lines to show the three dorsal tegumen processes; **Fig. 9** natural shape without flattening (phallus removed). — Illustrations not to the same scale.

© Entomologischer Verein Apollo e. V., Frankfurt am Main

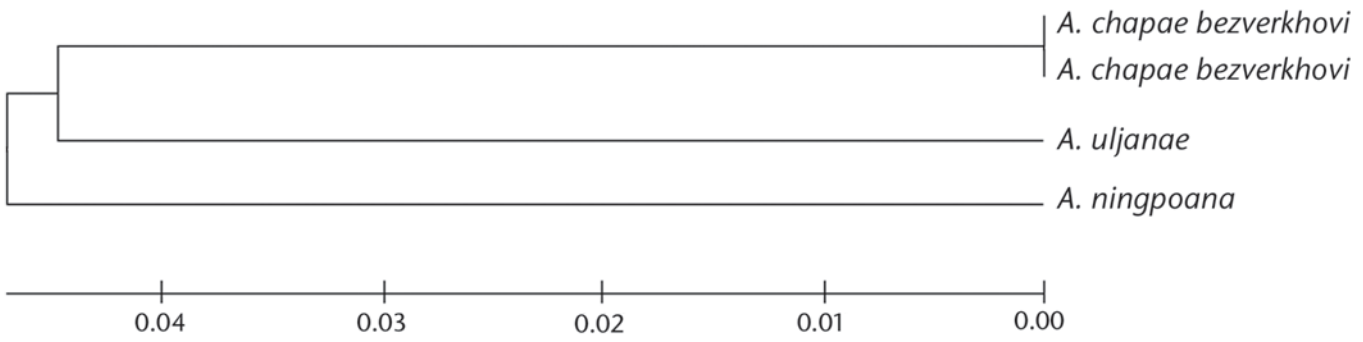

Fig. 10: Unrooted linearized similarity tree. Minimal evolution model (SBL = 0.138).

Analysis of molecular markers

The holotype and a paratype specimen of *A. c. bezverkhovi* have been screened for mitochondrial COI gene DNA to provide a "genetic passport". We could not find any other records for *A. chapae* in the public databases like BOLD (BOLD 2008) and NCBI GenBank (NCBI 2008). Both specimens involved in the study have, as may be expected, shown the same DNA marker sequence, see Fig. 10; the base sequences see in Tab. 2. Two other accepted species have been involved in analysis for comparison.

The questions concerning molecular phylogeny of *Actias* will be discussed in forthcoming publications. An unrooted linearized similarity tree is shown in Fig. 10.

Distribution

A. c. bezverkhovi was found in the northern part of the Langbian plateau with a narrow stripe of primary mountain forests at the elevation 1400–2200 m.

So far, no geobotanical investigation of this area was conducted, and no attempts have been made to find the natural hostplant(s) of *A. c. bezverkhovi*. Nevertheless, the analysis given below may help to define directions for future research. Generally it is well known that some *Actias* species, including the Chinese (from Nanling Shan) population of *Actias c. chapae,* have Pinaceae as their hostplants (Renner et al. 2006, Wu & Naumann 2006), and the spatial distribution of their populations may thus also be delimited by hostplant distribution. According to the review by Wang & Hong (2001) there are only a few original Vietnamese pine species.

The first, *Pinus dalatensis* de Ferré (Dalat or Vietnamese white pine), has a very restricted range in evergreen subtropical forests of Vietnam at elevations above 1500 m. Often it occurs in mixed stands being very sparsely distrubuted; the species' survival appears to be threatened.

The second, *Pinus fenzeliana* Hand.-Mzt. (Hainan white pine), is common in South China in Hainan, Guangxi and Guizhou provinces as well as in central Vietnam at elevations of 1000–1600 m.

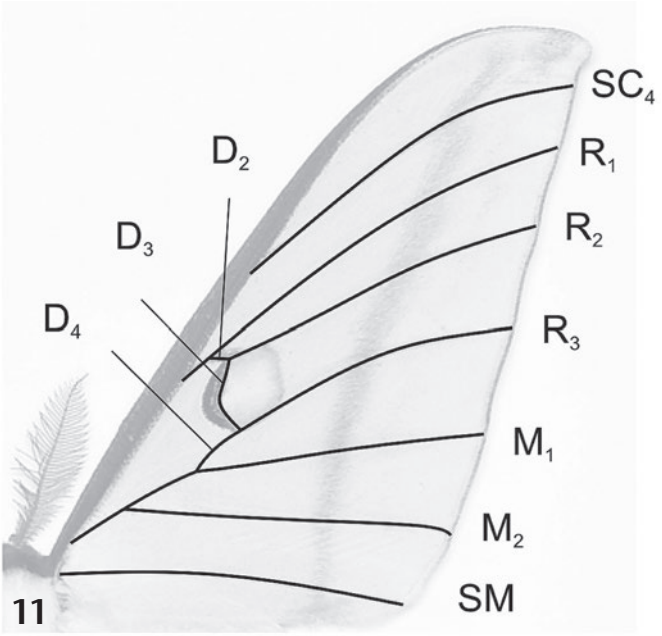

Fig. 11: Forewing of *Actias chapae,* with schematic venation to show vein D2 (N. Ivshin).

The third pine species is *Pinus kesiya* Royle ex Gordon, known as Langbian pine in Vientam. It is considered as very perspective species for planting and cultivation primarily due to the ease of planting and good adaptation. According to Razal et al. (2005), *P. kesiya* has very wide distribution. In the East it forms forests on the Philippines, within an altitudinal range of 750–2450 m. In Thailand, *P. kesiya* is confined to the Northwest and North, occurring mainly in the mountain ranges west and southwest of Chiang Mai to and across the border to Myanmar. Isolated occurrences are found to the northwest of Maung Nan in the northern mountains, on the Phu Kradung plateau near Loei, and the most southerly location in Thailand is in the Mieng Hills. The pine forms here the forests between 1000 and 1500 m, with the range of altitudinal distribution from 300–1800 m, although rarely below 800 m. In Vietnam, *P. kesiya* occurs in the Annam Cordillera mountain chain, which is in southern Vietnam. The best and most extensive stands are in the Langbian plateau near Dalat,

© Entomologischer Verein Apollo e. V., Frankfurt am Main

at a mean altitude of about 1500 m, although the species grows within the altitudinal range of 1300–2300 m on Langbian. Other locations in Vietnam are in the Haut Donnai plateau southeast of Dalat in the forest of Yankar, at Tourland To, and at P'sore north of Kinda. Small stands have also been reported on the southern mountains of Langbian, and also scattered in the semi-arid zones of the North, and further up in the highlands close to the border with China.

At lower elevations up to 1000 m occur *Pinus merkusii* Jungh & De Vriese and *Pinus massoniana* Lamb. (Goldammer 1992). They form the forests covering the area of 135 000 ha which are highly endangered by overlogging due to illegal logging, expanding shifting agriculture, grazing practices, and increasing demands for fuelwood and charcoal production. *P. merkusii* is considered as one of the few truly tropical pine species, occurring naturally in Southeast Asia including Myanmar, Thailand, Laos, Vietnam, Cambodia, Sumatra in Indonesia, and in the islands of Luzon and Mindoro in the Philippines. Either pure or mixed plantations of *P. kesiya* with *P. merkusii* and *P. massoniana* are reported to have been established in Vietnam.

Thus, it is to be expected that the natural hostplant of *A. chapae bezverkhovi* may be found among these 5 pine tree species.

Acknowledgments

The authors would like to express their gratitude to the following persons: our colleagues Pavel Oudovichenko and Yuri Bezverkhov for invaluable help and assistance in all stages of current research; Viktor Sinjaev for important information concerning biology and behaviour of *A. c. chapae*, for loan of material, rare publications and providing photographs of type specimens; Professor Dr. Ilia Zakharov and Dr. Elena Shaikevich for advising in genetic research and providing laboratory facilities. Further Dr. Stefan Naumann for loan of material and critical comments on the manuscript.

References

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994): DNA primers for amplification of mitochondrial cytochrome-c oxidase subunit I from diverse metazoan invertebrates. — Molecular Marine Biology and Biotechnology, New York, **3:** 294–299.

Goldammer, J. G. (1992): A fire problem analysis. — International Forest Fire News, New York, Geneva, **7:** 13–16.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003a): Biological identifications through DNA barcodes. — Proceedings of the Royal Society of London, series B, Biologcal Sciences, London, **270:** 313–321.

——, Ratsingham, S., & Dewaard, J. R. (2003b): Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. — Proceedings of the Royal Society of London, series B, Biologcal Sciences, London, **270:** 596–599.

Kumar, S., Tamura, K., & Nei, M. (2004): MEGA 3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. — Briefings in Bioinformatics, Oxford, **5:** 150–163.

Mell, R. (1950): Aus der Biologie der chinesischen *Actias* Leach (*Argema chapae* sp. n., *A. sinensis* f. *virescens* f. n.). — Entomologische Zeitschrift, Frankfurt am Main, **60** (6): 41–45, (7): 53–56.

Morishita, K., & Kishida, Y. (2000): Moths of Nanling mountains, Guangdong, S. China. — Yadoriga, Tokyo, **187:** 10–17, pls. 1–4 [in Japanese].

Nässig, W. A., & Brechlin, R. (1995): Designation of the lectotype of *Actias chapae* Mell, 1950 (Lepidoptera: Saturniidae). — Nachrichten des Entomologischen Vereins Apollo, Frankfurt am Main, N.F. **16** (2/3): 309–310.

Razal, R. A., Tolentino, E. L. T. jr., Carandang, W. M., Nghia, N. H., Hao P. S. & Luoma-Aho, T. (2005): Status of genetic resources of *Pinus merkusii* (Jungh & De Vriese) and *Pinus kesiya* (Royle ex Gordon) in Southeast Asia. — Los Baños, Philippines (UPLBCFNR), Malaysia (IPGRI-APO); 33 pp.

Renner, F., Prange, R., Plontke, R., & Nässig, W. A. (2006): Die Zucht des Hybriden *Actias sinensis* (Walker, 1855) Männchen × *A. dubernardi* (Oberthür, 1897) Weibchen (Lepidoptera: Saturniidae). — Nachrichten des Entomologischen Vereins Apollo, Frankfurt am Main, N.F. **27** (1/2): 29–52.

Wang H. & Hong J. (2001): Genetic resources, tree improvement and gene conservation of five-needle pines in East Asia. — IUFRO Working Party 2.02.15. International Conference, Medford, Oregon, USA (July 23–27, 2001): 73–78.

Wu Y. & Naumann, S. (2006): The preimaginal instars of *Actias chapae* (Mell, 1950) (Lepidoptera: Saturniidae). — Nachrichten des Entomologischen Vereins Apollo, Frankfurt am Main, N.F. **27** (1/2): 17–21.

Zakharov, I. A., Shaikevich, E. V., & Ivshin, N. V. (2007): The Barcode of Life and butterflies identification. — Priroda ("Nature", Russia), Moscow, **9:** 1–5.

Internet references

BOLD (2008): Barcode of Life data systems. — www.boldsystems.org/views/login.php (last accessed: 15. iv. 2008).

NCBI (2008): National Center for Biotechnology Information (GenBank). — www.ncbi.nlm.nih.gov (last accessed: 15. iv. 2008).

Received: 3. iv. 2008

© Entomologischer Verein Apollo e. V., Frankfurt am Main, August 2008 ISSN 0723-9912

© Entomologischer Verein Apollo e. V., Frankfurt am Main